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RESEARCH ARTICLE

EFFECTS OF THE AQUEOUS BARK EXTRACT FROM STRYCHNOS CAMPTONEURA AGAINST 40% ETHANOL-INDUCED MALE INFERTILITY IN MALE GUINEA PIGS (CAVIA PORCELLUS)

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Abstract

The overall objective of this study was to assess the effects of the aqueous extract derived from the bark of *Strychnos camptoneura* on reproductive functions in male guinea pigs (*Cavia porcellus*) following ethanol 40% intoxication. To assess these effects, 20 guinea pigs were randomly sorted into 4 lots, each containing 5 animals and given distilled water at a dose of 1ml/kg for lot 1; ethanol at a dose of 1ml/kg for lot 2 (positive controls); and ethanol at a dose of 1ml/kg for lot 3; ethanol at 1ml/kg and aqueous extract from *Strychnos camptoneura* trunk bark at 100mg/kg; and for lot 4: ethanol at 1ml/kg and aqueous extract from *Strychnos camptoneura* bark, given at a dosage of 250mg/kg. The results demonstrated that oral administration of aqueous extract from *Strychnos camptoneura* trunk bark (100mg/kg and 250mg/kg) in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40% moderately stimulates food consumption compared with positive controls given ethanol exclusively. Results demonstrated that oral administration of aqueous extract from *Strychnos camptoneura* trunk bark (100 and 250 mg/kg) in ethanol-treated male guinea pigs causes a non-significant ($P > 0.05$) increase in the weight of vas deferens, seminal vesicles and penis, compared with negative controls given distilled water and positive controls treated exclusively with ethanol. In addition, gavage of the animals at the doses studied produced a significant ($P < 0.01$) dose-dependent increase in testicular and epididymal weights, in contrast to the positive controls, which showed a significant ($P < 0.01$) decrease in these two sexual organs. Treatment with the extract did not result in significant changes ($P > 0.05$) in the weight of the vas deferens, seminal vesicles, or penis. Oral administration of the aqueous extract of *Strychnos camptoneura* trunk bark (100 and 250mg/kg) to ethanol-exposed male guinea pigs resulted in highly and highly significant ($p < 0.01$; $p < 0.001$) dose-

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dependent increases in sperm concentration per epididymal tail compared with lots treated with distilled water and ethanol alone. In addition, there was a highly significant ($p < 0.001$) dose-related increase in the rate of motile and vital spermatozoa, compared with negative and positive controls given distilled water and ethanol alone. In contrast, a highly significant ($p < 0.01$; $p < 0.001$) decrease in sperm count, motility rate and vitality was observed in male guinea pigs treated with ethanol alone. These results show that gavage of the animals with the extract, at the doses studied after exposure to ethanol, results in a non-significant ($p > 0.05$) increase in serum testosterone levels, compared with positive controls. In addition, positive ethanol-only controls were found to experience a significant ($p < 0.01$) decrease in testosterone levels when compared to negative controls receiving distilled water. These results provide evidence that the extract may offer protective benefits against alcohol-induced male infertility, confirming its androgenic and spermatogenic potential.

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Introduction:-

Reproduction is a biological process that enables the production of new organisms of a species from pre-existing individuals of the same species (Morère and Pujol, 2003). Its proper functioning is crucial for the continued existence of a species and the stability of a population. Any dysfunction in this process has social consequences at various levels (Ngom, 2010). Several parameters are involved in this process, including androgenic and spermatogenic parameters. Today, these two sexual parameters are undergoing real dysfunction due to multiple factors, including excessive alcohol consumption, which thus leads to male infertility (Ngoula et al., 2017; Kenfack et al., 2018; Dupont and Lévy, 2019; Lounis, 2020).

Indeed, alcohols, used in industry, domestic households and also consumed in the form of beverages, are highly reprotoxic. They cause impaired male fertility, affect the endocrine system and are often cited as factors contributing to poor sperm quality (Doreen et al., 2018). Alcohol consumption negatively affects the male reproductive system in a number of ways. Excessive consumption reduces sperm mobility, vitality and concentration, while increasing the number of malformed sperm. It also causes shrinkage of the testes and epididymis, and impairs testosterone production, leading to infertility, impotence, reduced libido and reduced secondary sexual characteristics (Saihia et al., 2015; Hoyer et al., 2020; Lounis, 2020). Faced with these adverse effects, and given the high cost of pharmaceutical products, local populations are turning to medicinal plants with androgenic and spermatogenic potential, including *Strychnos camptoneura*, the subject of our study.

Indeed, *Strychnos camptoneura* is a medicinal plant of the Loganiaceae family, widely used in traditional pharmacopoeia in Congo and other countries such as Cameroon, CAR, Liberia, etc. (Leeuwenberg, 1969; Leeuwenberg, 1980; Morabandza et al., 2016) for the treatment of sexual disorders, malaria, ulcers, inflammation, pain, diabetes, fever, microbial infections, hernia and parasitosis (Morabandza et al., 2016a; Morabandza et al., 2016b). A few studies have already been carried out on the efficacy of this plant to enhance sexual function in Wistar rats (Morabandza et al., 2017), on the evaluation of the androgenic potentialities of the hydroethanolic extract of the trunk barks of *Strychnos camptoneura* (Loganiaceae) in Wistar rats (Akassa et al., 2022), as well as on the spermatogenic and antiradical activities of the hydroethanolic extract of the trunk bark of *Strychnos camptoneura* (Loganiaceae) in Wistar rats (Akassa et al., 2022). However, no studies evaluating the effects of the aqueous extract of the trunk bark of *Strychnos camptoneura* (Loganiaceae) on reproductive functions following ethanol intoxication have been carried out. With this in mind, The primary goal of this study was to determine the effects of orally administered aqueous *Strychnos camptoneura* bark extract on reproductive functions in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Animal material

Male guinea pigs (*Cavia porcellus*), ranging in weight from 273 g to 500 g, were utilized for this investigation. These animals were procured from vendors at the Total market, located in Brazzaville's second arrondissement, Baongo. They were acclimatized for two weeks at the Biotechnology and Animal Physiology Laboratory of the

École Nationale Supérieure d'Agronomie et de Foresterie (ENSAF) before being subjected to the experiment. During this period, they were fed a standard laboratory diet and provided with water ad libitum.



Figure 1:- Male *Cavia porcellus*.

Plant material

The plant material used in this study was the bark of *Strychnos camptoneura* trees, collected from Mvoula, a village 740 km from Brazzaville, in the sub-prefecture of Itoumbi, Cuvette-Ouest department, northern Congo. The bark sample had already been authenticated at I.R.S.E.N (formerly ORSTOM Brazzaville) and registered under no. 271. The bark was dried at room temperature in the F.S.S.A. Biochemistry and Pharmacology Laboratory, then ground with a wooden mortar and sieved.



Figure 2:- *Strychnos camptoneura* barks (a) and powder (b).

Preparation of *Strychnos camptoneura* aqueous extract.

An aqueous extract was prepared from *Strychnos camptoneura* bark using the maceration method. 50 g of *Strychnos camptoneura* trunk bark powder was mixed with 500 ml of distilled water for 48 hours. The macerate was filtered through absorbent cotton, then evaporated using a Lab Companion hot plate. The resulting brown, dried extract was weighed and stored in a sealed container for future pharmacological evaluation.

40% Ethanol Preparation

Preparation of 40% ethanol was carried out in accordance with Gay-Lussac's alcohol wetting table. To obtain a final concentration of 40%, 104.01 ml of distilled water were added to 100 ml of 80% alcohol of initial concentration

Evaluation of the effects of aqueous extract of *Strychnos camptoneura* trunk bark on reproductive functions in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol

To assess the effects of the extract, twenty guinea pigs were randomly assigned to four lots of five animals each. Each lot received a specific treatment for a period of 32 days

- Group I: Positive and negative controls
 - lot 1 (negative controls): distilled water at a dose of 1 ml/kg ;
 - lot 2 (positive controls): ethanol at 1 ml/kg;
- Group II: Ethanol + Extract
 - Lot 3: ethanol at 1 ml/kg and aqueous extract from *Strychnos camptoneura* trunk bark at 100 mg/kg;
 - Lot 4: ethanol at 1 ml/kg and aqueous extract from *Strychnos camptoneura* trunk bark at 250 mg/kg.

Effects of aqueous extract from *Strychnos camptoneura* trunk bark on food consumption in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol

Diet is an essential factor influencing reproductive performance. It is therefore essential to control it in order to properly interpret the results. To assess the effect of the aqueous extract from *Strychnos camptoneura* trunk bark on food consumption in male guinea pigs intoxicated with 40% ethanol, A QUIGG brand balance (5000 g capacity, 1 g accuracy) was used to weigh the quantities of food distributed and refused for each lot on a daily basis over a 32-day period. Food consumption was determined by applying the following formula:

Quantity of feed consumed = Quantity of feed given - Quantity of feed refused.

Effects of aqueous extract from *Strychnos camptoneura* trunk bark on weight development in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The weight of each animal was recorded every other day over a 32-day period using a QUIGG brand balance with a 5000 g capacity and 1 g accuracy. This weight monitoring enables us to follow the evolution of the guinea pigs' weight under treatment, in order to detect any potential Effects from the aqueous extract of *Strychnos camptoneura* on their growth.

Effect of the aqueous extract from *Strychnos camptoneura* trunk bark on androgen-dependent sex organ weight in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The aim of this study was to evaluate the effect of this extract on the weight of androgen-dependent sex organs in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol. The size, weight and secretory functions of the androgen-dependent sex organs (testes, epididymides, seminal vesicles, vas deferens and penis) are regulated by androgens (Gayrard, 2007). Thus, after 32 days of treatment with 100 and 250 mg/kg doses of aqueous *Strychnos camptoneura* bark extract, the ethanol-intoxicated guinea pigs were euthanized in groups. After removal and degreasing, these organs (testes, epididymides, vas deferens, seminal vesicles and penis) were weighed using a branded precision balance (Mettler- Tdedo) of capacity (160g) and precision (10-3 g) (Akassa et al., 2019).

Effect of the bark aqueous extract from *Strychnos camptoneura* on microscopic characteristics of semen in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The effect of the aqueous extract of *Strychnos camptoneura* trunk bark on the microscopic characteristics of semen in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol were studied in order to assess the effects of the extract on sperm function (motility, vitality, sperm concentration). After 32 days of administration, the guinea pigs were sacrificed and the tail of the right epididymis of each guinea pig was harvested, excised and dilacerated in a Petri dish containing 10 ml of 0.9% NaCl solution,; then incubated in a water bath at 36°C (Akassa et al., 2019).

Effect of aqueous extract of *Strychnos camptoneura* trunk bark on sperm motility in male guinea pigs (*Cavia porcellus*) after 40% ethanol intoxication.

Sperm motility was assessed by direct examination. A 20 µl solution of the sample was placed between slide and coverslip, then observed at x40 magnification. Motile and immobile spermatozoa were counted on 12 randomly selected microscopic fields. The percentage of motile spermatozoa was determined using the formula proposed by Ngoula (2008):

$$\% \text{ percentage of motile spermatozoa} = \frac{\text{Number of motile spermatozoa}}{\text{total spermatozoa count}} \times 100$$

Effects of aqueous extract of *Strychnos camptoneura* trunk bark on sperm concentration in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Sperm counts were determined using a Thoma cell. Semen was diluted 100 times with a 35% formalin solution to immobilize the sperm cells before counting. 10 μ l of the diluted semen sample were placed in the Thoma cell chamber, which has a surface area of 0.2 mm² and a depth of 0.1 mm. A coverslip was then placed on top of the chamber. The sperm cells were counted under a Leica DM 750 microscope at a magnification of 40 times. The counting was performed in five large grid squares of the hemocytometer. The counting procedure was repeated two times, and the average of these two counts was used to calculate the number of sperm per epididymis tail, using the following formula:

$$\text{Number of sperm / tail} = \text{number of sperm / mm}^3 \times 1000 \times 10.$$

Where 10 is the volume of the solution in ml and 1000 the conversion factor from mm³ to ml (Ngoula, 2008; Akassa et al., 2019).

Effects of aqueous extract of *Strychnos camptoneura* trunk bark on sperm vitality in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

Sperm vitality was evaluated through a specific methodology involving the preparation of slides for counting live and dead spermatozoa, followed by staining and microscopic observation at 40x magnification. On smears, live spermatozoa are stained red by 2% eosin and dead ones are not stained (Landry et al., 2019).

Effect of aqueous bark trunk extract from *Strychnos camptoneura* on serum testosterone in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The objective of this study was to evaluate the effects of the extract on testosterone levels and to gain a comprehensive understanding of the hormonal composition of the aqueous extract derived from the trunk bark of *Strychnos camptoneura*. Androgens, whose main hormone is testosterone, are synthesized from cholesterol. These androgens have an anabolic effect, boosting protein synthesis and muscle mass, and contributing to testicular and epididymal enlargement (Gayrard, 2007). In guinea pigs, any increase in serum testosterone is accompanied by an increase in the secretory activity and weight of these organs (Gonzales, 2003).

Collection of serum and determination of serum testosterone.

24 hours after the last treatment with aqueous extract of *Strychnos camptoneura* trunk bark (100 and 250 mg/po), blood was collected from each animal in EDTA tubes. The serum collected after centrifugation five minutes later, was divided into the marked tubes and stored at -20°C for serum testosterone testing. This serum biochemical parameter was assayed at the Faculty of Health Sciences Laboratory.

Serum testosterone was determined using the Accu Diag TM ELISA Kit (Diagnostic Automation Inc).

Principle

The ELISA kit for testosterone determination employs a competitive binding principle. In this assay, both labeled and unlabeled testosterone molecules are present, and they compete to bind to a specific antibody. The extent of binding of the labeled testosterone to the antibody is inversely related to the concentration of unlabeled testosterone in the sample.

Statistical analysis of results

Results were entered and analyzed using a computerized tool. Variables were entered in Excel.

The collected data was analyzed using analysis of variance (ANOVA), Student's t-test, and Mann-Whitney U test to determine significant differences between the 'test' groups. Results are expressed as mean \pm standard error, with $p < 0.05$ as the significance threshold.

Results:-**Effect of aqueous trunk bark extract from *Strychnos camptoneura* on food consumption in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.**

Figure 3 shows the effects of the aqueous extract from *Strychnos camptoneura* trunk bark on food consumption in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol. The results in this figure show that the

aqueous extract of *Strychnos camptoneura* trunk bark moderately stimulates food consumption in treated guinea pigs in a dose-dependent manner compared with positive controls.

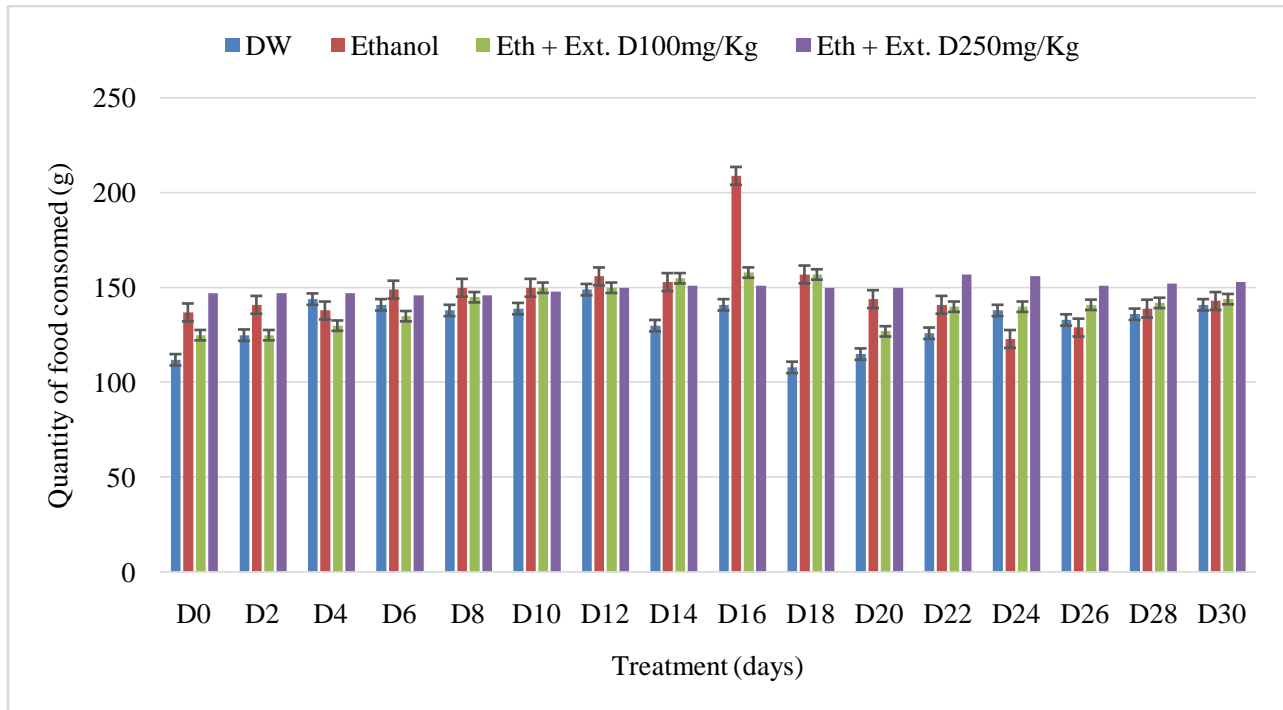


Figure 3:- Effect of aqueous extract of *Strychnos camptoneura* trunk bark on food consumption in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

Effect of aqueous trunk bark extract from *Strychnos camptoneura* on weight growth in male guinea pigs (*Cavia porcellus*) after intoxication with ethanol 40%.

Figure 4 shows the effect of aqueous bark extract from *Strychnos camptoneura* on weight gain in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol. As shown in the figure, daily oral administration of the aqueous extract of *Strychnos camptoneura* trunk bark at doses of 100 mg/kg and 250 mg/kg to male guinea resulted in a non-significant increase in body weight when compared to the ethanol-treated control group.

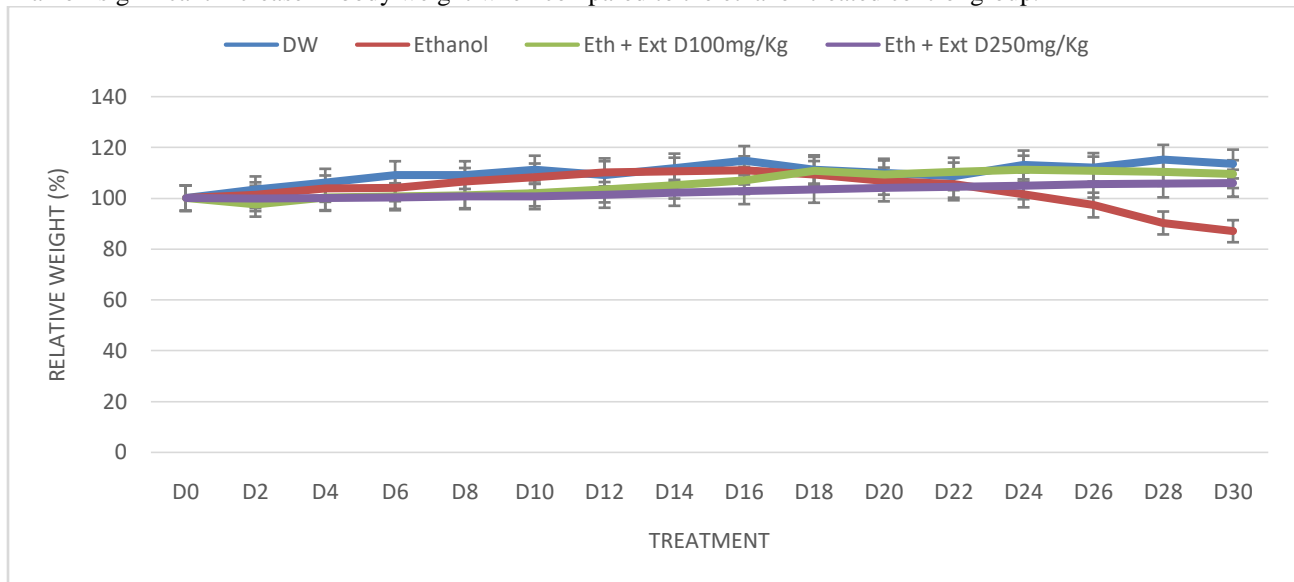


Figure 4:- Effect of aqueous extract from *Strychnos camptoneura* trunk bark on body weight growth in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Effect of aqueous extract from *Strychnos camptoneura* trunk bark on androgen-dependent sex organ weights in male guinea pigs (*Cavia porcellus*) after ethanol 40% intoxication

Table 1 summarizes the effect of the aqueous extract of *Strychnos camptoneura* trunk bark against 40% ethanol-induced male infertility on androgen-dependent sex organ weights in male guinea pigs (*Cavia porcellus*).

The table shows that administration of aqueous extract of *Strychnos Camptoneura* trunk bark to guinea pigs by the oral route at dependent doses (100 and 250 mg/kg/in.) resulted in a non-significant increase ($P > 0.05$) in the weight of vas deferens, seminal vesicles and penis, compared with those treated with ethanol. It also significantly ($P < 0.01$) increased testicular and epididymal weights in male guinea pigs treated with both doses, compared with those treated with ethanol. Consequently, a non-significant decrease ($P > 0.05$) in testicular and epididymal weights was observed, as well as a non-significant increase ($P > 0.05$) in the weights of vas deferens, seminal vesicles and penis in male guinea pigs treated with ethanol compared to those given distilled water.

Table 1:- Effect of aqueous extract of *Strychnos camptoneura* trunk bark against 40% ethanol-induced male infertility on androgen-dependent sex organ weights in male guinea pigs (*Cavia porcellus*).

androgeno- dependant Organs (g)	Treatments			
	Distilled Water(1ml/kg)	Ethanol (1ml/kg)	Ethanol (1ml/kg) + S.c. (100mg/kg)	Ethanol(1ml/kg) + S.c. (250mg/kg)
Testes	0.19±0.007	0.15±0.02*	0.27±0.04*	0.25±0.03*
Epididymes	0.04±0.0009	0.01±0.005*	0.03±0.001*	0.03±0.002*
vas deferens	0.02 ±0.007	0.01±0.002ns	0.01±0.004ns	0.01±0.001ns
Seminal vesicles	0.20 ±0.003	0.19±0.02ns	0.20±0.05ns	0.21±0.04ns
Penis	0.18±0.002	0.17±0.02ns	0.16±0.08ns	0.17 ±0.01ns

Values are means ± MSE, with n = 3, ns : $P > 0.05$ non-significant difference from controls (distilled water and ethanol); *: $P < 0.01$ significant difference; DW: distilled water; Sc: *Strychnos camptoneura*

Effect of aqueous extract from *Strychnos camptoneura* trunk bark on serum testosterone in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The effect of the aqueous extract of *Strychnos Camptoneura* trunk bark on serum testosterone is summarized in figure 5. Oral administration of the aqueous extract of *Strychnos camptoneura* trunk barks in guinea pigs at the doses studied (100 and 250 mg /kg) causes a non-significant ($p > 0.05$) increase in serum testosterone levels compared with ethanol-treated positive controls. However, the ethanol-only positive controls showed a significant decrease ($p < 0.01$) in testosterone levels compared with the negative controls (distilled water).

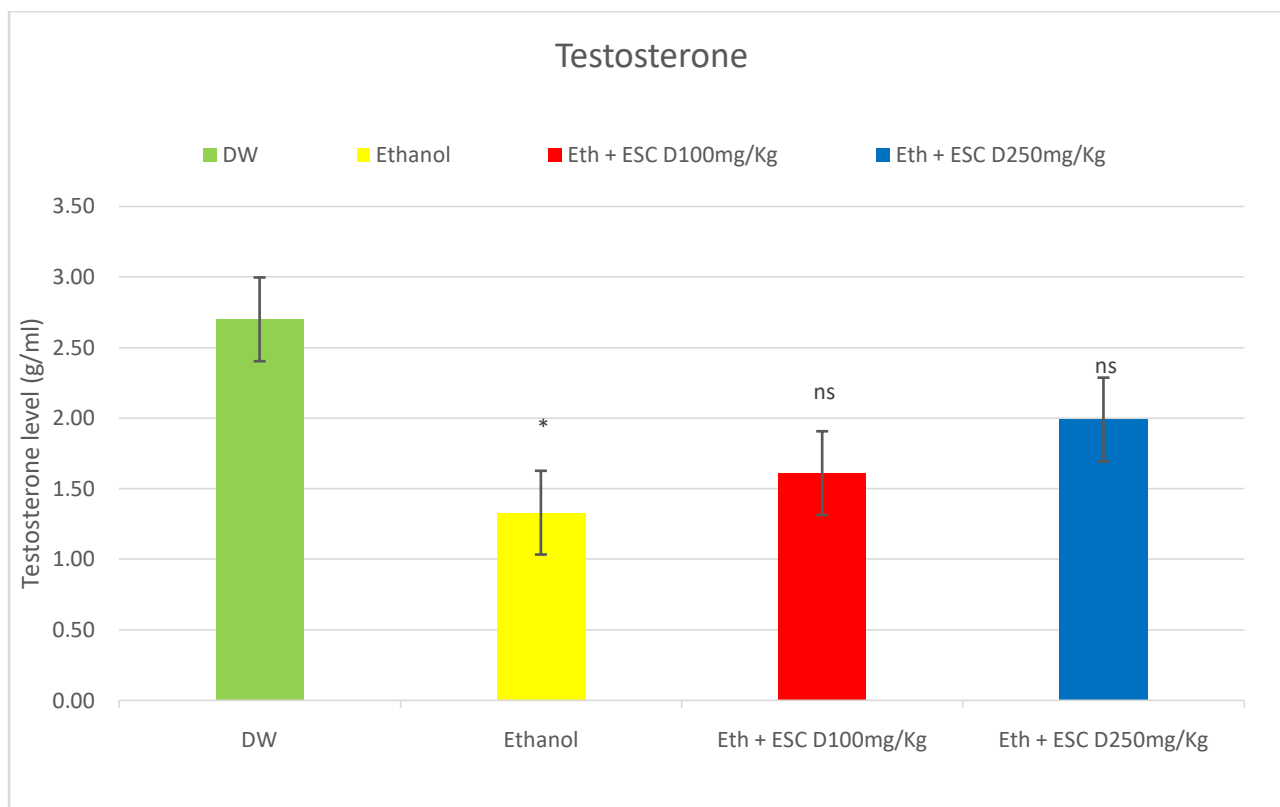


Figure 5:- Effects of aqueous extract from *Strychnos camptoneura* trunk bark on serum testosterone in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Effects of *Strychnos camptoneura* trunk bark aqueous extract on semen microscopic characteristics (sperm concentration, motility and vitality) in male guinea pigs (*Cavia porcellus*) after 40% ethanol intoxication.

Table 2 shows the effects of the aqueous extract of *Strychnos camptoneura* trunk bark on the microscopic characteristics of spermatozoa in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol. The table shows that administration of the aqueous extract of *Strychnos camptoneura* trunk bark (doses 100 and 250mg/kg) orally to guinea pigs produced highly significant increases ($p < 0.001$) in sperm concentration, motility and vitality in male guinea pigs treated with both doses (100 and 250mg/Kg), compared with ethanol-treated positive control guinea pigs. However, highly significant decreases ($p < 0.01$) in sperm concentration and motility and highly significant decreases ($p < 0.001$) in sperm vitality were observed in ethanol-treated male guinea pigs compared with distilled water-treated negative controls.

Table 2:- Effects of aqueous extract from *Strychnos camptoneura* trunk bark on semen microscopic characteristics in male guinea pigs (*Cavia porcellus*) after 40% ethanol intoxication.

Microscopic characteristics of the semen	Treatments				
	Distilled (1ml/kg)	Water	Ethanol (1ml/kg)	Ethanol (1ml/kg)+S.c. (100mg/kg)	Ethanol (1ml/kg) + S.c. (250mg/kg)
Concentration (10^6)	9.66±0.33		5.66±0.66 **	13±0.57 ***	11.33±0.88 ***
Mobility (%)	48.33±1.66		38.33±1.66**	61.66±1.66 ***	56.66±1.66 ***
Vitality (%)	43.33±1.66		26.66±1.66***	66.66±1.66 ***	45±2.88 ***

Values are means ± MSE, with n = 3, **: P < 0.01 and ***: P < 0.001 significant difference from positive controls ; DW: distilled water ; S.c.: *Strychnos camptoneura*.

Discussion:-

The overall objective of this study was to assess the effects of the aqueous trunk bark extract of *Strychnos camptoneura* on reproductive functions in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Effect of aqueous trunk bark extract from *Strychnos camptoneura* on food consumption in male guinea pigs (*Cavia porcellus*) after intoxication with 40% ethanol.

Administration of the aqueous bark extract from *Strychnos camptoneura* at the doses studied in male guinea pigs (*Cavia porcellus*) after ethanol intoxication moderately stimulated food consumption in guinea pigs compared with ethanol-treated positive controls, suggesting that the aqueous extract of the trunk bark of *Strychnos camptoneura* had a stimulating effect on guinea pig appetite. This moderate stimulation could be explained by the fact that the animals were intoxicated with ethanol, which suppresses their appetite by having a negative effect on the satiety center.

Effect of aqueous extract from *Strychnos camptoneura* trunk bark on weight growth in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

One of the specific aims of this study was to evaluate the effects of aqueous extract of *Strychnos camptoneura* trunk bark on weight growth in male guinea pigs (*Cavia porcellus*) after 40% ethanol intoxication. The results of this investigation reveal that daily oral administration of aqueous extracts from *Strychnos camptoneura* trunk bark (100mg/kg and 250mg/kg) to guinea pigs for 32 days resulted in a non-significant increase in body weight over time compared with ethanol-treated positive controls. This non-significant increase in body weight is explained by the guinea pigs' chronic alcohol intoxication, which resulted in moderate food consumption. These results are similar to those obtained by Akomolafé et al, (2017) on the effects of aqueous extract of *Tetracarpidium conophorum* leaves on ethanol-induced male infertility in male rats, which resulted in a significant increase in body weight. This difference between the two studies would be due either to the different types of plant organs used, the concentration of the solvent, the chemical composition and concentration of various secondary metabolites contained in the extract, or the extraction doses and techniques used (Wacho et al., 2017 and Akassa et al., 2019).

Furthermore, guinea pigs in the positive control lot that received ethanol only, experienced a non-significant decrease in body weight compared to those in the negative control lot that received distilled water. This reduction in body weight would therefore be due to the effect of alcohol, which leads to a shrinkage and reduction in the weight of the testes and epididymis (Saihia et al., 2015; Hoyer et al., 2020; Lounis, 2020; Mokrani and Hidouci, 2022).

Effect of aqueous extract of *Strychnos camptoneura* trunk bark on androgen-dependent sex organ weights in male guinea pigs (*Cavia porcellus*) after 40% ethanol intoxication.

Administration of the aqueous extract of the trunk bark of *Strychnos camptoneura* at the doses studied in male guinea pigs (*Cavia porcellus*) after ethanol intoxication resulted in a non-significant increase ($P > 0.05$) in the relative weights of the seminal vesicles and penis, and no significant variation in the weight of the vas deferens compared with those treated with ethanol. These results are similar to those obtained by Akono et al (2007), who noted no significant variation in the relative weight of testes and reproductive appendages in rats treated with methanol extract of *Basella alba*. The non-conformity of these results could be due either to the type of solvent and plant organs used, or to the chemical composition and concentration of various secondary metabolites contained in the extract, or to the extraction doses and techniques used (Wacho et al., 2017 and Akassa et al., 2019).

In contrast, a significant increase ($P < 0.01$) in the relative weight of testes and epididymides was observed in the extract-treated groups compared to the ethanol-treated positive control guinea pigs. The increase in testicular weight could be due to increased biosynthesis of the androgens required for testicular development, growth and function. These results suggest that the aqueous extract of *Strychnos camptoneura* trunk bark has an ameliorative effect on the relative weight of normal testes and epididymides, as well as those affected by the reprotoxic action of alcohol. Our results concur with those of Mokrani and Hidouci, (2022) on the evaluation of the effect of spinach extract after the reprotoxic and hepatotoxic action of alcohol, which stipulate that spinach supplementation improves testicular and epididymal weight in response to the deleterious effects of alcohol-induced oxidative stress.

As a result, a non-significant decrease ($P > 0.05$) in testicular and epididymal weights was observed, as well as a non-significant increase ($P > 0.05$) in seminal vesicle and penile weights in male guinea pigs treated with ethanol compared to those given distilled water. These results can be explained by the work carried out by Widenius et al, (1989) and Adler, (1992) who showed that excessive ethanol consumption can lead to male hypofertility, characterized by a decline in certain secondary characteristics and testicular shrinkage.

This result confirms our hypothesis that the aqueous extract of *Strychnos camptoneura* trunk bark increases the relative weight of androgen-dependent sex organs.

Effect of aqueous extract of *Strychnos camptoneura* trunk bark on microscopic characteristics of semen in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

The study conducted on the microscopic characteristics of semen after administration of aqueous extract of *Strychnos camptoneura* trunk bark (doses 100 and 250mg/kg) orally to guinea pigs produced highly significant ($p < 0.001$) increases in sperm concentration, motility and vitality in male guinea pigs treated with both doses (100 and 250mg/Kg) compared with ethanol-treated positive controls. Our results are similar to those obtained by Lounis, (2020) on the evaluation of the effects of aqueous extract of spinach on male fertility after ethanol intoxication in rabbits which showed a significant increase in sperm concentration, motility and vitality in spinach-treated rabbits. They would also be close to those found by Ogwo et al, (2016) who found a significant increase in sperm concentration and motility in rats treated with ethanolic extract of *Pausinystalia yohimbe*. Similarly, a significant increase in sperm concentration and motility was observed by Syed et al., (2018) in rats treated with ethanolic extracts of *Chlorophytum*. In addition, Mokrani and Hidouci, (2022) showed that the use of aqueous spinach extract, had significant effects on sperm concentration and motility in male rabbits. These differences were attributed to the type of drugs administered and their chemical composition (Akassa et al., 2023).

However, highly significant ($p < 0.01$) decreases in sperm concentration and motility and very highly significant ($p < 0.001$) decreases in sperm vitality were observed in ethanol-treated male guinea pigs compared with negative controls treated with distilled water. These results concur with those conducted by Saihia et al, (2015); Hoyer et al, (2020) and Lounis, (2020) who showed that excessive alcohol consumption triggers a decrease in sperm motility, vitality and concentration. This result confirms the hypothesis that the aqueous extract of *Strychnos camptoneura* trunk bark improves the microscopic characteristics of semen.

Effect of aqueous extract of *Strychnos camptoneura* trunk bark on serum testosterone in male guinea pigs (*Cavia porcellus*) after ethanol intoxication 40%.

Oral administration of the aqueous extract of *Strychnos camptoneura* trunk bark in guinea pigs at the doses studied (100 and 250 mg /kg) caused a non-significant ($p > 0.05$) increase in serum testosterone levels compared with ethanol-treated positive controls. This non-significant increase in serum testosterone levels suggests that this extract has steroidogenic activity, acting on the hypothalamic-pituitary-testicular complex that stimulates testosterone synthesis by Leydig cells. According to Akassa et al, (2019), this increase is due to the presence in the extract of phenolic compounds and flavonoids with androgenic properties. Our results are close to those obtained by Akassa et al, (2022) who showed that the ethanolic extract of *Strychnos camptoneura* led to a significant increase in serum and testicular testosterone levels in rats. They are also similar to those reported by Talla et al. (2021), who reported significant increases in serum testosterone levels in rats treated with methanolic extract of *Ficus asperifolia*. This difference is thought to be due to the level of solvents and plant organs used. Furthermore, positive controls treated with ethanol alone showed a significant decrease ($p < 0.01$) in testosterone levels compared to negative controls (distilled water). This decrease in testosterone levels could be due to the direct effect of ethanol on the gonads, which reduces testosterone concentration. These results are in line with those of Mokrani and Hidouci, (2022) who assert that excessive and prolonged alcohol consumption has adverse effects on spermatogenesis, as it inhibits testosterone synthesis. Similarly, Widenius et al, (1989), Adler, (1992) have shown that acute and chronic exposure to ethanol is associated with decreased levels of the hormones GnRH and LH, leading to reduced testosterone synthesis and consequent testicular atrophy.

Conclusion:-

The results of this study show that oral administration of the aqueous extract of *Strychnos camptoneura* trunk bark to male guinea pigs (*Cavia porcellus*) after ethanol intoxication (40%) moderately stimulates food consumption, improves weight growth and significantly increases the relative weight of the testes and epididymides. The extract also improves the microscopic characteristics of the semen. In addition, administration of this extract to guinea pigs at the doses studied produced a non-significant increase in serum testosterone levels. The results of this study suggest that the aqueous extract of *Strychnos camptoneura* trunk bark may have protective effects against alcohol-induced male infertility, thus confirming its potential to enhance androgenic and spermatogenic activity.

Conflict of interest

The authors declare that they have no competing interests.

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